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Potentiation by vasopressin of adrenergic vasoconstriction in the rat isolated mesenteric artery

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1 The aim of the present study was to investigate in rat mesenteric artery rings whether low concentrations of vasopressin could modify the contractile responses to noradrenaline and electrical stimulation of perivascular nerves.

2 Vasopressin ($10^{-10}-10^{-7}$ M) caused concentration-dependent contractions ($pD_2 = 8.36 \pm 0.09$). The V_1-receptor antagonist $d(CH_2)_5$Tyr(Me)AVP ($10^{-9}-10^{-8}$ M) produced parallel rightward shifts of the control curve for vasopressin. Schild analysis yielded a pA_2 value of 9.83 with a slope of 1.10 ± 0.14.

3 Vasopressin (3×10^{-10} and 10^{-9} M) caused concentration-dependent potentiation of the contractions elicited by electrical stimulation (2–8 Hz; 0.2 ms duration for 30 s) and produced leftward shifts of the concentration-response curve for noradrenaline. The V_1-receptor antagonist induced concentration-dependent inhibitions of potentiation induced by vasopressin. The selective V_1-receptor agonist [Phe^2, Orn^8]-vasotocin (3×10^{-10} and 10^{-9} M) induced potentiation of electrical stimulation-evoked responses which was also inhibited in the presence of the V_1 antagonist (10^{-8} M). In contrast, the V_2-receptor agonist deamino-8-D-arginine vasopressin (desmopressin $10^{-8}-10^{-7}$ M) did not modify the electrical stimulation-induced responses and the V_2-receptor antagonist [$d(CH_2)_5$, D-Ile^2, Ile^4, Arg^8]-vasopressin ($10^{-8}-10^{-7}$ M) did not affect the potentiation evoked by vasopressin.

4 In artery rings contracted by 10^{-6} M noradrenaline in the presence of 10^{-6} M guanethidine and 10^{-6} M atropine, electrical stimulation (2, 4 and 8 Hz) produced frequency-dependent relaxations which were unaffected by 10^{-9} M vasopressin but abolished by 10^{-6} M tetrodotoxin.

5 Vasopressin also potentiated contractions elicited by KCl and contractions induced by addition of $CaCl_2$ to KCl depolarized vessels. The augmenting effects were inhibited by the V_1 antagonist.

6 In the presence of the calcium antagonist nifedipine (10^{-6} M), vasopressin failed to enhance the contractile responses to electrical stimulation, noradrenaline and KCl.

7 The results demonstrate that low concentrations of vasopressin strongly potentiate the contractions to adrenergic stimulation and KCl depolarization. This effect appears to be mediated by V_1 receptor stimulation which brings about an increase in calcium entry through dihydropyridine-sensitive calcium channels.

Keywords: Rat mesenteric artery; V_1 and V_2 vasopressin receptors; vasopressin antagonists; adrenergic nerve stimulation

Introduction

Vasopressin causes powerful constriction in a variety of vascular regions (Nakano, 1973; Altura & Altura, 1977; Valloton *et al*., 1990). The vasopressin receptor designated V_1 seems to mediate the vasoconstrictor action of the peptide, whereas its antidiuretic action is mediated by a adenosine 3′:5′-cyclic monophosphate (cyclicAMP)-dependent mechanism coupled to V_2 receptors (Michell *et al*., 1979; Penit *et al*., 1983; Thibonnier, 1988). Further studies of the physiological effects of vasopressin have implicated a possible role of V_2 receptors in mediating vasodilatation in some vascular beds. Administration of the selective V_2 agonist, deamino-8-D-arginine vasopressin (desmopressin), or vasopressin in the presence of a selective V_1 receptor antagonist, decreased peripheral vascular resistance in both man and dogs (Liard & Spadone, 1984; Bichet *et al*., 1988; Tagawa *et al*., 1995) and produced relaxation of human and rat isolated arteries (Yamada *et al*., 1993; Martínez *et al*., 1994a,b).

Vasopressin may also modify the effects of other vasoactive substances that are found in plasma or released from perivascular nerves. Several studies have demonstrated a significant augmentation of the vasoconstricting actions of catecholamines (Bartelstone & Nasmyth, 1965; Karmazyn *et al*., 1978; Guc *et al*., 1992). However, there are other studies in which vasopressin did not change the constrictor activity of the peripheral sympathetic nervous system (Hilgers *et al*., 1993) or even attenuate (Harada *et al*., 1991) the pressor action of catecholamines. Moreover, it is not clear whether the direct and indirect vascular effects of vasopressin are mediated by the same receptor site. Thus the present study was designed to investigate whether low concentrations of vasopressin could modify the constrictor response obtained by noradrenaline, potassium chloride and stimulation of perivascular nerves supplying rat mesenteric arteries. We also determined whether the modulating effect of vasopressin on vascular responsiveness depends on the activation of V_1- or V_2-receptors.

Methods

Male Wistar rats weighing 300 to 400 g were killed by cervical dislocation and exsanguinated. Second and third generation branches of the superior mesenteric artery were dissected from the mesentery. The arteries were immediately placed in chilled Krebs-Henseleit solution and cleaned of surrounding tissue. Rings 3 mm long were cut for isometric recording of tension. Two stainless steel pins 100 μm in diameter were introduced through the arterial lumen of the ring. One pin was fixed to the wall of the organ bath, while the other was connected to a force-displacement transducer (Grass FT03). Changes in isometric force were recorded on a Grass polygraph (model 7). Each artery ring was set up in a 4 ml bath containing modified Krebs-Henseleit solution of the following composition (mM):

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NaCl 115, KCl 4.6, $MgCl_2.6H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25; glucose 11.1 and disodium EDTA 0.01. The solution was equilibrated with 95% O_2 and 5% CO_2 to give a pH of 7.3–7.4. Temperature was held at 37°C. To establish the resting tension for maximal force development, a series of preliminary experiments was performed on artery rings of similar length and outer diameter which were exposed repeatedly to 60 mM KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 10 mN. The artery rings were allowed to attain a steady level of tension during a 2 h equilibration period before testing. Functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10^{-7}–10^{-6}M) during contraction obtained with noradrenaline (10^{-6}–3×10^{-6} M).

Following the equilibration period, concentration-response curves for vasopressin (10^{-10}–10^{-7} M) were obtained in paired rings under resting tension in the absence and presence of the V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP (10^{-9}–10^{-8} M). To determine whether the response to vasopressin differs from that to a selective V_1 agonist, concentration-response curves for [Phe^2, Orn^8]-vasotocin were obtained in the absence and presence of the V_1 antagonist (10^{-8} M).

Electrical field stimulation was provided by a Grass S88 stimulator (Grass Instruments, Quincy, M.A., U.S.A.) via two platinum electrodes positioned on each side and parallel to the axis of the artery ring. To asses the nature of the contractile responses and avoid direct stimulation of smooth muscle, frequency-response relationships were determined on a group of arteries in the presence and absence of 10^{-6} M tetrodotoxin, following a procedure previously described (Duckworth *et al*., 1989; Aldasoro *et al*., 1993). In summary, the protocol was designed to find the optimal stimulation parameters (15 V, 0.2 ms duration) causing a contractile response that was completely eliminated by 10^{-6} M tetrodotoxin. Frequency-response relationships were determined with 30 s trains of pulses at 2, 4 and 8 Hz. A period of 10 min was allowed between stimulations.

To study the effects of experimental substances on electrical field stimulation-induced contractions, we performed the following protocol: after an initial set of stimulations (2, 4 and 8 Hz) at 10 min intervals, another set of stimulations was given in the presence or absence of experimental substances. The drugs tested included vasopressin (10^{-10} to 10^{-9} M), the selective V_1 vasopressin agonist [Phe^2,Orn^8]-vasotocin (10^{-10} to 10^{-9} M), the V_2 vasopressin agonist desmopressin (10^{-8} to 10^{-7} M), the V_1 receptor antagonist d(CH_2)$_5$Tyr(Me)AVP (10^{-10} to 10^{-8} M), the V_2 receptor antagonist [d(CH_2)$_5$, D-Ile^2, Ile^4,Arg^8]-vasopressin (10^{-8} to 10^{-7} M), and the reuptake blocker cocaine (10^{-6} M). In control arterial rings, repeated electrical stimulation revealed less than 10% variability in the magnitude of the contractions over a period of two hours. Antagonists were added to organ bath chambers 15 min before the initiation of frequency- or concentration-response relationship.

In another series of experiments neurogenic stimulation (2, 4 and 8 Hz, 15 V, 0.2 ms duration, 30 s trains at 10 min intervals) was performed in ring segments contracted with noradrenaline (10^{-6} M) in the presence of atropine (10^{-6} M) and guanethidine (10^{-6} M) to reveal the possible existence of nonadrenergic-noncholinergic neurogenic relaxation that has been observed in mesenteric resistance vessels of rat (Kawasaki *et al*., 1988; 1990). In the same preparations electrical stimulation was carried out in the presence of vasopressin (3×10^{-10} M) to check the possibility that vasopressin may impair neurogenic relaxation. At the end of each series, tetrodotoxin (10^{-6} M) was added and electrical stimulation was repeated to confirm the neurogenic nature of the response.

Concentration-response curves for noradrenaline and KCl were determined in a cumulative manner. Control (in the absence of vasopressin) and experimental (in the presence of vasopressin) data were obtained from separate vascular preparations. Another group of artery rings was incubated with the V_1 antagonist before exposure to noradrenaline or KCl. When KCl was used, prazosin (10^{-6} M) was added to the organ bath in order to prevent activation of α-adrenoceptors by noradrenaline released by neuronal depolarization.

To study the effects of vasopressin on calcium-induced contractile responses, a group of artery rings was incubated in calcium-free solution containing 25 or 100 mM KCl. After a 30 min washout period, concentration-response curves to $CaCl_2$ (10^{-6} to 3×10^{-3} M) were determined in paired rings in the absence and presence of either vasopressin (3×10^{-10} M) or vasopressin together with the V_1 receptor antagonist (10^{-8} M).

In another group of experiments, the preparations were preincubated with the Ca^{2+} channel blocker nifedipine for 20 min before the addition of vasopressin.

Drugs

The following drugs were used: tetrodotoxin, nifedipine, prazosin hydrochloride, noradrenaline hydrochloride, acetylcholine chloride, arginine vasopressin acetate salt, guanethidine, [(1- (β-mercapto-β, β-cyclopentamethylenepropionic acid)-2-(O-methyl)-tyrosine, 8-arginine) vasopressin] (d(CH_2)$_5$Try (Me)AVP), deamino-8-D-arginine vasopressin (desmopressin), atropine (Sigma Chemical Co, St. Louis, MO, U.S.A.); [Phe^2, Orn^8]-vasotocin, [d(CH_2)$_5$,D-Ile^2,Ile^4,Arg^8]-vasopressin (Peninsula Laboratories Europe, Merseyside, U.K.) and cocaine chlorhydrate (Abelló, Madrid, Spain). All drugs were dissolved in Krebs solution except nifedipine, which was dissolved initially in ethanol and further diluted in Krebs solution to the final concentration. Drugs were added to the organ bath in volumes of less than 70 μl. Stock solutions of the drugs were freshly prepared each day and kept on ice throughout the experiment.

Data analysis

The data are expressed as means ± s.e.mean. pD_2 (negative logarithm of the molar concentration at which half-maximum contraction occurs) was determined from individual concentration-response curves by non-linear regression analysis. The pA_2 values for V_1 vasopressin receptor antagonist were determined from a Schild plot (Arunlakshana & Schild, 1959). The concentration-ratios (CR) were calculated as the ratio between the EC_{50} value for vasopressin in the presence and absence of different concentrations of the antagonist. A Schild plot was constructed with the CRs: log (CR-1) (ordinate scale) was plotted against log (antagonist concentration) (abscissa scale) and pA_2 was estimated as the intercept of the regression line with the abscissa scale (Arunlakshana & Schild, 1959). Relaxation is expressed as the percentage of relaxation from precontraction in response to noradrenaline (10^{-6} M). In each experimental group n indicates the number of animals. Differences between agonist and antagonist-treated groups were assessed by one-way analysis of variance (ANOVA). Differences between groups were identified by t test. Statistical significance was accepted at $P<0.05$.

Results

Effects of vasopressin

Vasopressin (10^{-10}–10^{-7} M) caused concentration-dependent contractions with a pD_2 of 8.36 ± 0.09. The presence of the V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP (10^{-9}–10^{-8} M) in the organ bath induced significant shifts ($P<0.05$) of the control curve to the right in a concentration-dependent manner, with no change in the maximum response (Figure 1a). Schild analysis of these data yielded a pA_2 value of 9.83 with a slope of 1.10 ± 0.14 indicating competitive antagonism.

The selective V_1 receptor agonist [Phe^2,Orn^8]-vasotocin (10^{-10}–10^{-7} M) induced concentration-dependent contractions in all arteries tested. Maximal responses and pD_2 values were equivalent to those obtained with vasopressin (Figure 1b). The V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP (10^{-8} M) produced a parallel, rightward shift of the control curve (150 fold)

which did not differ from that obtained when vasopressin was the agonist (148 fold).

Effects of vasopressin on electrical stimulation-induced contractions

Electrical stimulation induced frequency-dependent increases in tension in all the experiments which were abolished by tetrodotoxin (10^{-6} M), guanethidine (10^{-6} M) and prazosin (10^{-6} M), thus indicating that the effect was due to the release of noradrenaline from adrenergic nerves acting on α_1-adrenoceptors.

Vasopressin 10^{-10} M did not change the contractions to electrical stimulation at the frequencies used (2, 4 and 8 Hz). At higher concentrations (3×10^{-10} and 10^{-9} M), vasopressin caused potentiation of the electrically-evoked responses (Figure 2a and b).

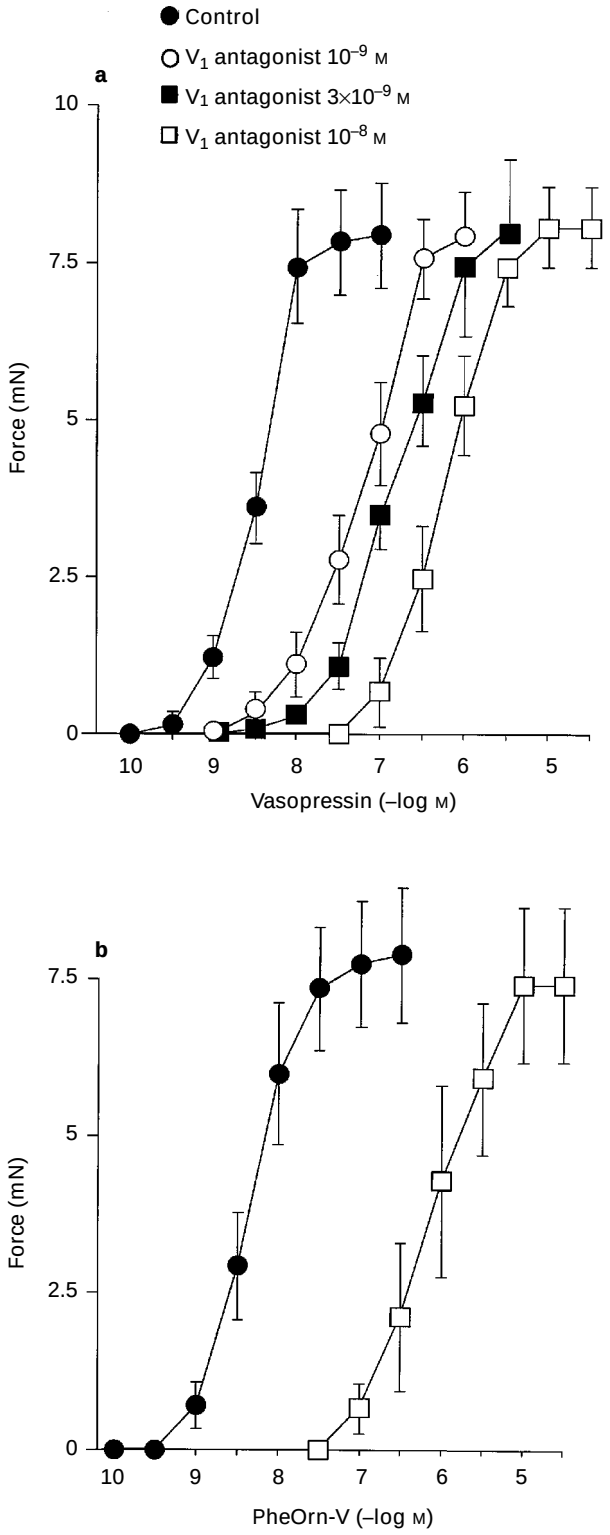

Figure 1 (a) Concentration-response curves for vasopressin in the absence ($n=10$) and in the presence of V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP (10^{-9}, 3×10^{-9} and 10^{-8} M) ($n=6$). (b) Concentration-response curves for the V_1 agonist (PheOrn-vasotocin) in the absence and presence of 10^{-8} M d(CH_2)$_5$Tyr(Me)AVP ($n=7$). Values are shown as the mean and vertical lines indicate s.e.mean.

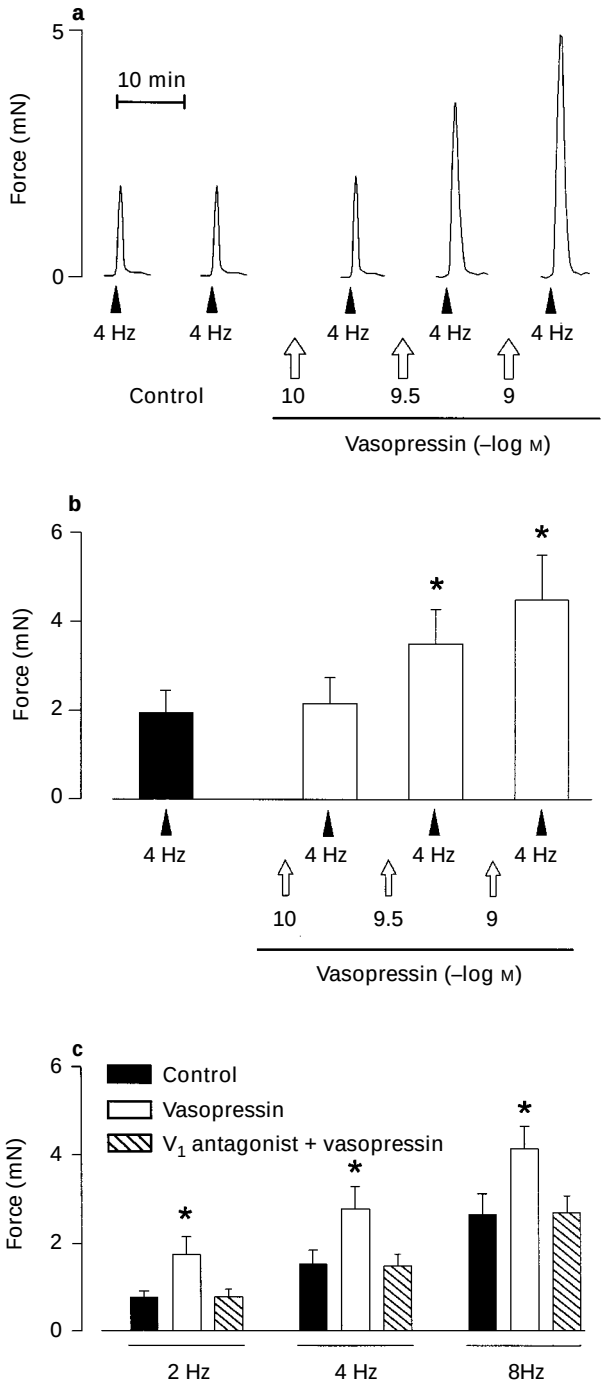

Figure 2 (a) Original tracings of contractile responses to field electrical stimulation (4 Hz) of rat mesenteric artery under control conditions and after incubation with various concentrations of vasopressin (10^{-10} to 10^{-9} M). (b) Contractile responses to electrical stimulation (4 Hz) in the absence and in the presence of vasopressin (10^{-10} to 10^{-9} M) ($n=9$). (c) The effects of 3×10^{-10} M vasopressin on frequency-dependent contractile responses to electrical field stimulation in the absence and presence of the V_1 antagonist d(CH_2)$_5$Tyr(Me)AVP (10^{-8} M) ($n=10$). Values in (b) and (c) are means $\pm$ s.e.mean; $^*P<0.05$ versus control.

The V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)AVP (10^{-9}–10^{-8} M) did not change control responses to electrical stimulation. Mean values of responses to stimulation at 2, 4 and 8 Hz in the absence of V_1 antagonist were 0.76 ± 0.15 mN, 1.52 ± 0.31 mN and 2.62 ± 0.45 mN, respectively ($n=5$). The presence of the V_1 antagonist (10^{-8} M) did not change significantly ($P>0.05$) the values for neurogenic contraction (0.77 ± 0.16 mN, 1.47 ± 0.26 mN and 2.65 ± 0.37 mN). However, the V_1 antagonist prevented the amplifying effect of vasopressin at all the frequencies used (Figure 2c).

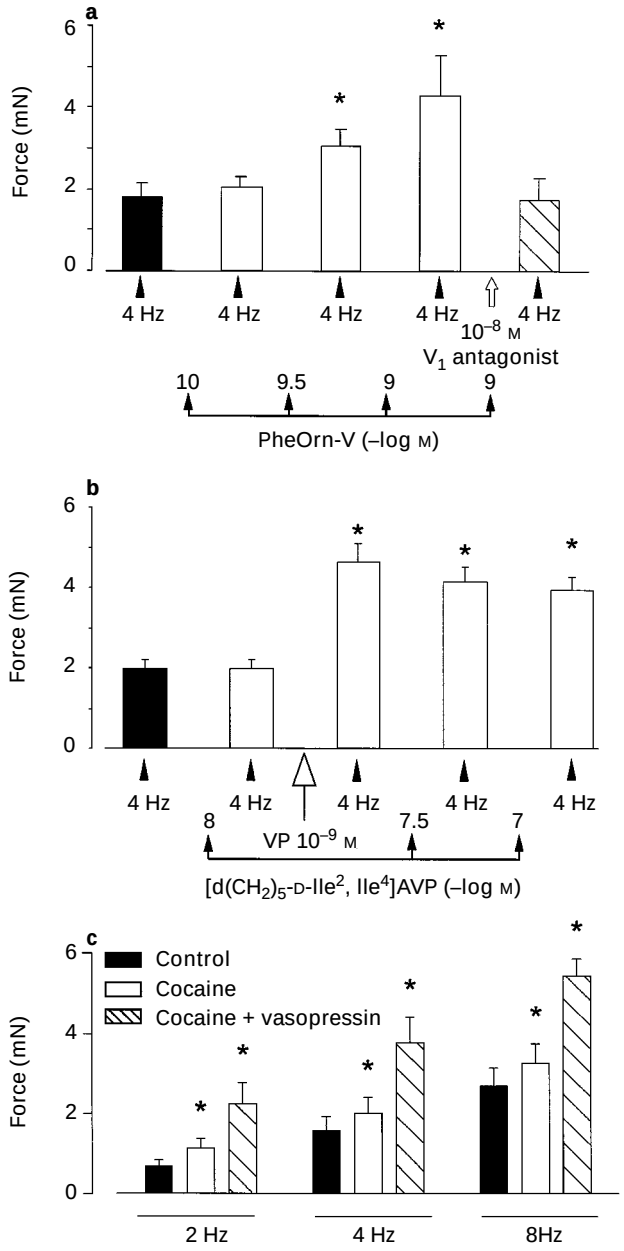

Figure 3 (a) Bar graph of contractile responses to electrical stimulation (4 Hz) in the absence and presence of increasing concentrations of the V_1 agonist [PheOrn-vasotocin] (10^{-10} to 10^{-9} M) ($n=9$). The presence of the V_1-receptor antagonist d$(CH_2)_5$Tyr(Me)AVP (10^{-8} M) abolished the augmentation of the contraction to electrical stimulation. (b) Contractile responses to electrical stimulation in the absence and presence of increasing concentrations of the V_2-receptor antagonist [d$(CH_2)_5$, D-Ile^2,Ile^4,Arg^8]-AVP (10^{-8}–10^{-7} M) ($n=9$). The potentiation induced by vasopressin (VP, 10^{-9} M) was not affected in the presence of the V_2-receptor antagonist. (c) Frequency-response relationship (2, 4 and 8 Hz) in the absence and presence of either cocaine (10^{-6} M) or cocaine together with vasopressin (3×10^{-10} M) ($n=6$). Values are means$\pm$s.e.mean; $^*P<0.05$ versus control.

The selective V_1 receptor agonist [Phe^2,Orn^8]-vasotocin (3×10^{-10} and 10^{-9} M) induced potentiation of electrical stimulation-evoked responses of a magnitude similar to that observed in the presence of the same concentrations of vasopressin. This potentiation was also inhibited in the presence of the V_1 antagonist (10^{-8} M) (Figure 3a).

To determine whether V_2 receptors are involved in the effects of vasopressin on electrical field stimulation, frequency-response relationships were obtained in the absence and presence of the V_2 receptor antagonist [d$(CH_2)_5$, D-Ile^2,Ile^4,Arg^8]-vasopressin (10^{-8}–10^{-7} M). The V_2 antagonist (10^{-8}–10^{-7} M) did not affect the control frequency-response relationship. In addition, the potentiation induced by vasopressin (10^{-9} M) was not modified ($P>0.05$) in the presence of increasing concentrations of the V_2 antagonist (Figure 3b). On

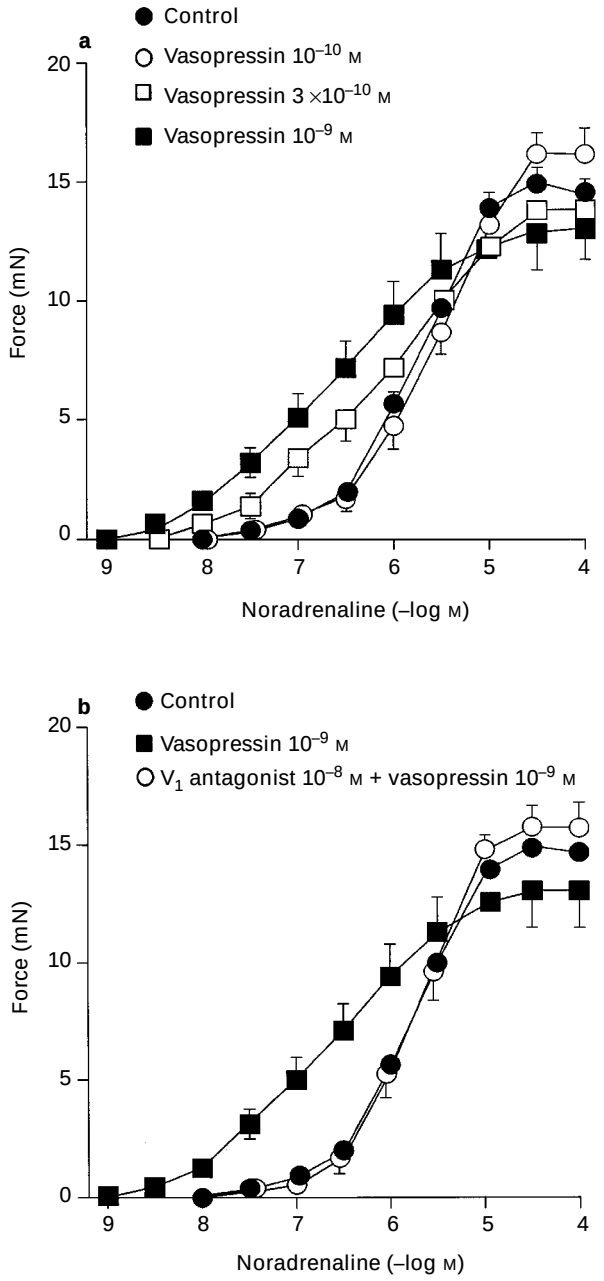

Figure 4 (a) Contractile effects of noradrenaline in the absence and presence of vasopressin (10^{-10} M, 3×10^{-10} M and 10^{-9} M) ($n=10$). (b) Concentration-response curves to noradrenaline in the absence and presence of either 10^{-9} M vasopressin or vasopressin (10^{-9} M) together with the V_1 antagonist d$(CH_2)_5$Tyr(Me)AVP (10^{-8} M) ($n=10$). Vertical lines show s.e.mean.

the other hand, the selective V_2-receptor agonist desmopressin (10^{-8}–10^{-7} M) did not change neurogenic contractions ($P>0.05$; $n=9$) (results not shown).

Blockade of neuronal catecholamine reuptake by cocaine (10^{-6} M) increased the contractile response to electrical field stimulation ($P<0.05$). In the presence of cocaine the contractile responses to electrical field stimulation were significantly enhanced by vasopressin (3×10^{-10} M) to an extent similar to that observed in the absence of cocaine (Figure 3c).

Effect of vasopressin on neurogenic relaxation

Electrical stimulation induced frequency-dependent relaxations of arterial rings contracted with noradrenaline (10^{-6} M) in the presence of guanethidine (to inhibit adrenergic neurotransmission) and atropine (to block muscarinic cholinergic receptors). Mean values of responses to electrical stimulation at 2, 4 and 8 Hz in the absence of vasopressin were $22.2\pm3.5\%$, $30\pm4.4\%$ and $35.3\pm3.0\%$, respectively ($n=7$). The presence of vasopressin (10^{-9} M) did not modify significantly ($P>0.05$) the values for neurogenic relaxation ($20\pm2.1\%$, $29.7\pm3.6\%$ and $32.7\pm3.6\%$) ($n=7$). However, 10^{-6} M tetrodotoxin abolished the relaxation induced by electrical stimulation (results not shown).

Table 1 pD_2 values and maximal contractions (E_{max}) to noradrenaline alone (control), in the presence of either vasopressin or the V_1 antagonist together with vasopressin

	pD_2	E_{max} (mN)
Control ($n=10$)	5.8 ± 0.1	14.7 ± 0.6
With vasopressin		
10^{-10} M ($n=10$)	5.7 ± 0.1	14.4 ± 1
3×10^{-10} M ($n=10$)	6.4 ± 0.2*	12.8 ± 1
10^{-9} M ($n=10$)	6.7 ± 0.1*	12.7 ± 1.6
With V_1 antagonist + vasopressin (10^{-9} M)		
10^{-9} M ($n=10$)	6.1 ± 0.1*	12.8 ± 1.5
3×10^{-9} M ($n=10$)	6.0 ± 0.2	14.1 ± 1.1
10^{-8} ($n=7$)	5.7 ± 0.1	14.9 ± 0.6

Values are means ± s.e.means. n number of rats. *$P<0.05$, versus arterial rings from control rats.

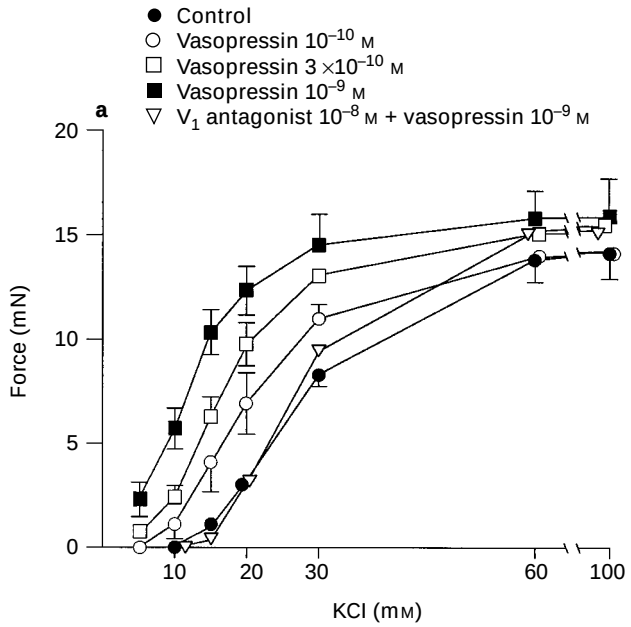

Figure 5 Concentration-response curves to KCl in the absence ($n=10$) and presence of vasopressin (10^{-10} M, 3×10^{-10} M and 10^{-9} M) ($n=7$). Previous addition of V_1-receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ (10^{-8} M) ($n=7$) inhibited the potentiation induced by 10^{-9} M vasopressin. Contractile responses to KCl were obtained in the presence of prazosin (10^{-6} M). Values are means and vertical lines show s.e.mean.

Effect of vasopressin on noradrenaline- and KCl-induced contractions

Vasopressin potentiated noradrenaline-induced contractions in a concentration-dependent manner (Figure 4a). The noradrenaline pD_2 values and maximal response in the presence and absence of vasopressin are shown in Table 1. The V_1 receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ produced a parallel, rightward shift of the potentiating effects of 10^{-9} M vasopressin on the noradrenaline concentration-response curve (Figure 4b). At 10^{-8} M the V_1 inhibitor brought the pD_2 values to values similar to those obtained in the noradrenaline control curve (Table 1).

The contractions to KCl were also significantly potentiated in a concentration-dependent-manner by vasopressin. The pD_2 changed from 1.55 ± 0.01 ($n=10$) in control to 1.77 ± 0.05 ($n=7$) ($P<0.05$) in the presence of 10^{-9} M vasopressin. Previous addition of V_1 antagonist (10^{-8} M) ($n=7$) inhibited the vasopressin-induced potentiation of the KCl response curve (Figure 5).

Vasopressin and calcium

The contractions to vasopressin were unchanged in the presence of the dihydropyridine calcium antagonist nifedipine (10^{-6} M); the pD_2 and maximum contraction were 8.36 ± 0.09 and 7.6 ± 0.9 mN ($n=10$), respectively, in the absence, and

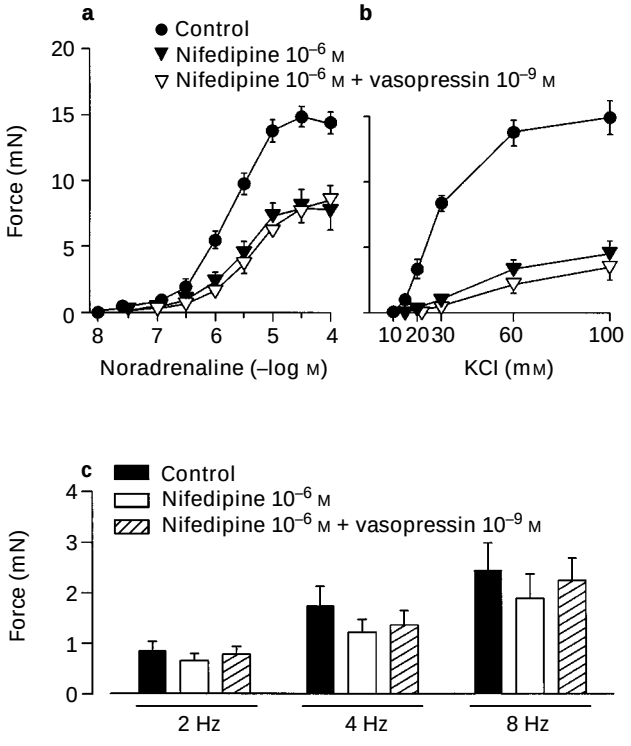

Figure 6 (a) Concentration-response curves to noradrenaline in the absence and presence of either nifedipine (10^{-6} M) or nifedipine with vasopressin (10^{-9} M) ($n=7$). (b) Concentration-response curves to KCl in the absence and presence of either nifedipine (10^{-6} M) or nifedipine with vasopressin (10^{-9} M) ($n=7$). (c) Frequency-response relationship in control artery rings and in artery rings treated with nifedipine (10^{-6} M) or nifedipine plus vasopressin (10^{-9} M) ($n=8$). Values are means and vertical lines show s.e.mean.

8.44 ± 0.07 and 7.0 ± 0.6 mN ($n=9$), respectively, in the presence of nifedipine.

Nifedipine (10^{-6} M) diminished significantly maximal responses to noradrenaline ($n=7$) and KCl ($n=7$) (Figure 6a and b) but did not change significantly the frequency-response curve to electrical stimulation (Figure 6c). In the presence of nifedipine (10^{-6} M), vasopressin (10^{-9} M) failed to enhance the constrictor response to noradrenaline, KCl and electrical stimulation (Figure 6).

In arteries incubated in Ca^{2+}-free solution containing 25 mM KCl, addition of calcium chloride ($10^{-6}-3\times10^{-3}$ M) elicited a concentration-dependent contractile response (Figure 7a). In the presence of vasopressin (3×10^{-10} M) maximal responses to $CaCl_2$ were increased but pD_2 values were not significantly different (3.77 ± 0.09 ($n=7$) versus 3.61 ± 0.05 ($n=7$)) ($P>0.05$). In vessels exposed to calcium-free solution containing 100 mM KCl, vasopressin induced a significant leftward shift of the concentration-response curve (Figure 7b). The pD_2 values were increased in the presence of vasopressin (3.88 ± 0.08 ($n=7$) versus 4.1 ± 0.08 ($n=7$)) ($P<0.05$). In the presence of the V_1 antagonist (10^{-8} M) the potentiation induced by vasopressin on the calcium chloride response curves was abolished.

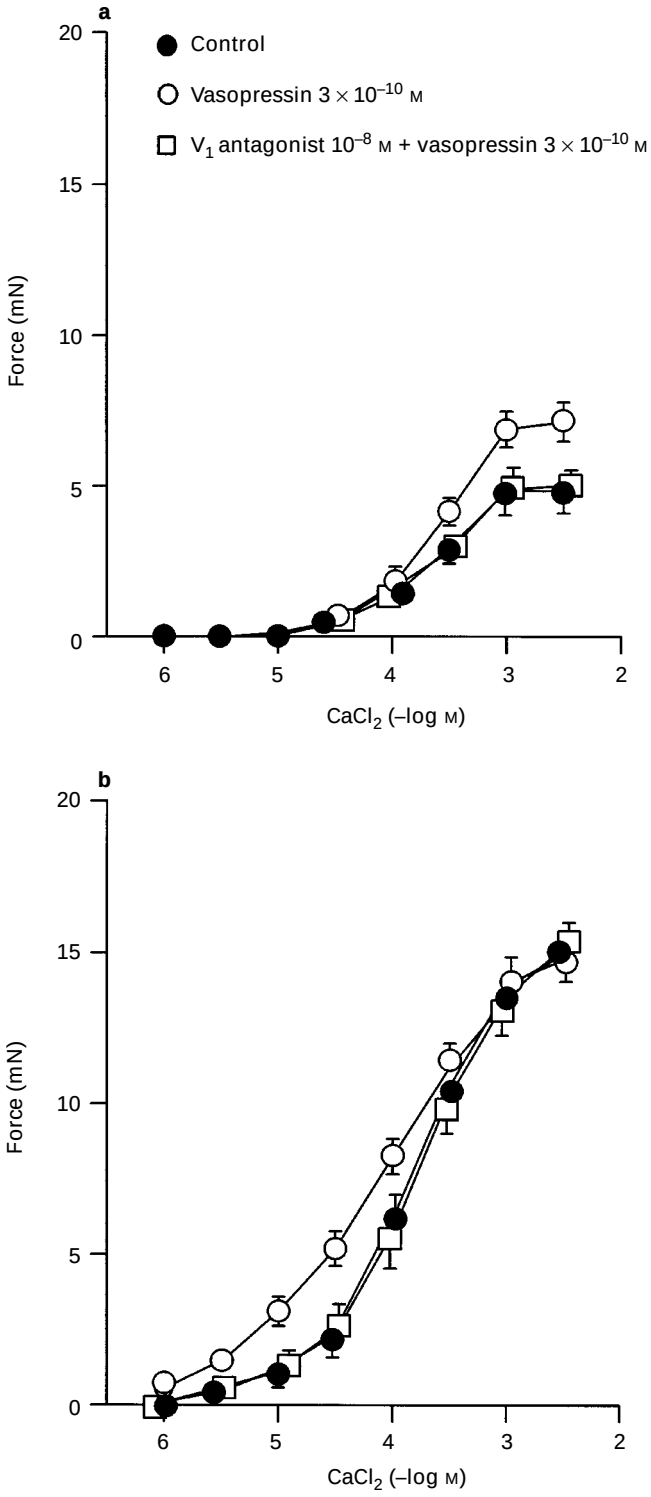

Figure 7 Contractions of the rat isolated mesenteric artery induced by the addition $CaCl_2$ to Ca^{2+}-free depolarizing solutions containing either 25 mM KCl (a) or 100 mM KCl (b). Concentration-response curves to $CaCl_2$ were repeated in presence of 3×10^{-10} M vasopressin and a combination of 3×10^{-10} M vasopressin and 10^{-8} M $d(CH_2)_5$Tyr(Me)AVP. The responses shown are the mean of 7 observations; vertical lines show s.e.mean.

Specificity of V_1 antagonist

The effect of $d(CH_2)_5$Tyr(Me)AVP on contractile responses was also studied in artery rings challenged with increasing concentrations of noradrenaline or KCl. The pD_2 values for noradrenaline ($n=10$) and KCl ($n=10$) in the absence of vasopressin were 5.8 ± 0.1 and 1.55 ± 0.01, respectively. These values were not significantly different ($P>0.05$) in the presence of V_1 antagonist (5.7 ± 0.1 and 1.54 ± 0.01; $n=7$). In addition, there was no significant difference in the pD_2 values for vasopressin in the absence (8.36 ± 0.09; $n=14$) and presence (8.44 ± 0.06; $n=5$) of 10^{-6} M prazosin.

Discussion

The results of the present study confirm previous findings showing that vasopressin is primarily a constrictor of mesenteric vascular smooth muscle due to V_1 vasopressin receptor stimulation (Ohlstein & Berkowitz, 1986; Vanner *et al.*, 1990; Martínez *et al.*, 1994b). Maximal responses and pD_2 values for vasopressin were similar to those obtained for [Phe^2, Orn^8]-vasotocin, a selective V_1 receptor agonist. In addition, the selective V_1 receptor antagonist $d(CH_2)_5$Tyr(Me)AVP inhibited the vasopressin contraction in a competitive way over a given concentration range of the antagonist. Schild analysis showing unitary slopes and antagonist pA_2 values obtained from these data indicate that the receptors involved in vasopressin-induced contraction belong to the classical V_1 receptor (Sawyer & Manning, 1985). Similar pA_2 values for the same V_1 antagonist have been found in human uterine arteries (Jovanovic *et al.*, 1995) and in several vascular beds of the rabbit (García-Villalón *et al.*, 1996).

The present study also showed that low concentrations of vasopressin enhance the contractile effects of electrical stimulation, noradrenaline and KCl depolarization. The electrical stimulation evoked responses were neurally mediated, because they could be readily abolished by tetrodotoxin (10^{-6} M). The resulting contraction observed was apparently mediated, to a great extent, by the release of the adrenergic transmitter, which in turn activates the α_1-adrenoceptor, since the response was inhibited by guanethidine and prazosin. The potentiating effects occur at vasopressin concentrations substantially lower than those required to produce a clear direct contractile response. In the presence of 10^{-9} M vasopressin the contractions to electrical stimulation almost doubled in amplitude at all the frequencies of stimulation used (2, 4 and 8 Hz). In addition, exposure to 10^{-9} M vasopressin, which elicited 7% of maximal noradrenaline contraction, resulted in a 8.5 fold leftward shift of the control curve to noradrenaline at the EC_{50} level.

Previous studies have shown that vasopressin may induce dilatation in some vascular beds (Schwartz *et al.*, 1985; Hirsch *et al.*, 1989; Martínez *et al.*, 1994a,b). Therefore we examined the potential role of V_2 receptor stimulation in the enhancing effects of vasopressin. The results do not support the intervention of V_2 receptors in these responses. First, the selective

V_2 agonist desmopressin did not modify responses to electrical field stimulation. On the other hand, the V_2 receptor antagonist [d$(CH_2)_5$, D-Ile^2, Ile^4, Arg^8]-vasopressin did not affect the potentiation induced by vasopressin. In contrast, our results showed that the selective V_1 receptor antagonist d$(CH_2)_5$Tyr(Me)AVP inhibited the potentiating effects of vasopressin on electrical field stimulation and noradrenaline-induced contractions in a concentration-dependent manner. In addition, the selective V_1 receptor agonist [Phe^2, Orn^8]-vasotocin induced potentiating effects similar to those observed in the presence of vasopressin. Therefore the results exclude a role for V_2 receptors in the potentiating effects of vasopressin and they are consistent with the hypothesis that V_1 receptor stimulation by vasopressin in the absence of direct contraction is followed by enhancement of responses to both endogenous and exogenous noradrenaline.

The mechanism of the increased responses induced by vasopressin is not readily apparent. It is conceivable that the effects of vasopressin on electrical field stimulation contractions could involve an effect on adrenergic nerves leading to release of noradrenaline or alternatively, vasopressin could act with noradrenaline at postjunctional receptor sites. Because noradrenaline release was not measured in this study, a contribution of presynaptic facilitating effects cannot be excluded. The fact that the concentration-response curve to vasopressin was not modified by prazosin suggests that the action of this peptide does not involve release of noradrenaline. The possibility that vasopressin could block the reuptake of noradrenaline and therefore enhance the contractile response is unlikely since the potentiating effects were still evident in the presence of cocaine. Alternatively, vasopressin-induced potentiation could be due to alterations at the receptor level leading to an increased affinity of noradrenaline for its receptor. However, this mechanism cannot explain the potentiation observed in KCl contractions. Thus potentiating effects of vasopressin do not seem to be restricted to events triggered by one specific receptor, but seem to reflect a general modification of the contractile function of vascular smooth muscle.

The relaxation observed in response to neurogenic stimulation after inhibition of adrenergic and cholinergic transmission, and the effective blockade of the relaxation by tetrodotoxin, confirm the presence of nonadrenergic, noncholinergic vasodilator nerves previously demonstrated in the perfused mesenteric bed of the rat (Kawasaki *et al*., 1988; 1990). We considered the possibility that the increase in neurogenic contractions induced by vasopressin could be due to impairment of nonadrenergic, noncholinergic relaxation. The results show that the highest concentration of vasopressin used (10^{-9} M) did not modify the vasodilator responses. Therefore, it appears that the amplifying effect of vasopressin, shown in our experiments, does not involve inhibition of neurogenic relaxation.

We also considered the possibility that stimulation of V_1 receptors may facilitate calcium entry through dihydropyridine calcium channels. It has been proposed that the vasoconstrictor effect induced by binding of vasopressin to its V_1 receptor is coupled to an increase in inositol phosphate metabolism and intracellular rise in calcium (Michell *et al*., 1979; Doyle & Rüegg, 1985; Standley *et al*., 1991). There is also evidence that vasopressin can stimulate a receptor-operated calcium influx pathway in rat aortic smooth muscle cells (Wallnöfer *et al*., 1987). Our results show that nifedipine abolished the potentiating effect of vasopressin on electrical field stimulation-induced contractions indicating that vasopressin potentiates only the component of the adrenergic contraction sensitive to nifedipine, but does not affect the dihydropyridine-insensitive component. In agreement with this, vasopressin failed to enhance the responses to noradrenaline and KCl when nifedipine was also present. Moreover, vasopressin augmented contractions elicited by $CaCl_2$ in KCl depolarized arteries, an effect completely reversed by V_1 receptor blockade. Thus it appears that potentiating effects of vasopressin result from an increase in calcium entry through voltage-dependent calcium channels. This probably takes place by the binding of vasopressin to the V_1 receptor, since the potentiating effects on $CaCl_2$ response curves were reversed by d$(CH_2)_5$Tyr(Me)AVP. Our results in isolated arteries provide support for recent observations demonstrating that vasopressin potentiates an L-type Ca^{2+} channel current in guinea-pig ventricular myocytes via V_1 receptor stimulation (Zhang *et al*., 1995). A similar change in the L-type Ca^{2+} channel has been previously described in urinary bladder smooth muscle cells of the guinea-pig (Bonev & Isenberg, 1992).

In conclusion, the results of the present study demonstrate that vasopressin, in addition to its vasoconstrictor effect, strongly potentiates the responses to electrical field stimulation, noradrenaline and KCl depolarization through activation of V_1 receptors. The potentiation is related to activation of the dihydropyridine-sensitive calcium channels.

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